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Genetic diversity and structure of two endangered mole salamander species of the Trans-Mexican Volcanic Belt

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Abstract

The most important factor leading to amphibian population declines and extinctions is habitat degradation and destruction. To help prevent further extinctions, studies are needed to make appropriate conservation decisions in small and fragmented populations. The goal of this study was to provide data from the population genetics of two micro-endemic mole salamanders from the Trans-Mexican Volcanic Belt. Nine microsatellite markers were used to study the population genetics of 152 individuals from two *Ambystoma* species. We sampled 38 individuals in two localities for *A. altamirani* and *A. rivulare*. We found medium to high levels of genetic diversity expressed as heterozygosity in the populations. However, all the populations presented few alleles per locus and genotypes. We found strong genetic structure between populations for each species. Effective population size was small but similar to that of the studies from other mole salamanders with restricted distributions or with recently fragmented habitats. Despite the medium to high levels of genetic diversity expressed as heterozygosity, we found few alleles, evidence of a genetic bottleneck and that the effective population size is small in all populations. Therefore, this study is important to propose better management plans and conservation efforts for these species.

Key Words

endemic species, endangered species, conservation genetics, microsatellite, *Ambystoma*, Nevado de Toluca Volcano, Sierra de las Cruces

Introduction

In Mexico, the Trans-Mexican Volcanic Belt (TMVB; Fig. 1A) is one of the most ecologically disturbed regions of the country and is highly fragmented by urban settlements, roads and agricultural areas (Sunny et al. 2017; González-Fernández et al. 2018). Also, the TMVB is the most important region in terms of endemic amphibian and reptile species and the second most important in terms of the number of species (Flores-Villela and Canseco-Márquez 2007). However, it is also one of the most disturbed areas of Mexico, with 44.7% of the TMVB highly fragmented by agricultural lands, 3.4% by urban settlements and 25.3% by roads. These anthropogenic activities are creating small patches of natural habitats and this scenario could start reducing the genetic flow between the populations of amphibians and reptiles of the TMVB (Lowe et al. 2005; Honnay and Jacquemyn 2007; Herrera-Arroyo et al. 2013).

This loss of genetic connectivity can decrease genetic diversity and increase the interpopulation genetic di-

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vergence, while increasing inbreeding levels and loss of alleles due to genetic drift (Young et al. 1996; Frankham et al. 2005; Lowe et al. 2005; Honnay and Jacquemyn 2007). This loss results in fluctuations of effective population size and other demographic and environmental parameters that can drive biological populations towards extinction (Gibbs 1998; Newman and Tallmon 2001; Johansson et al. 2006). Genetic diversity is highly important because it shapes the ability of populations to respond to environmental changes (Templeton et al. 1990; Frankham 1996; Frankham et al. 2003; Reed and Frankham 2003). Therefore, the International Union for the Conservation of Nature (IUCN 2017) has recognized genetic diversity as one of the three levels of biological diversity necessary to conserve species diversity (McNeely et al. 1990; Frankham 1998; IUCN 2017).

Amphibians are a key taxonomic group that is an ecological indicator as they are highly sensitive to habitat degradation and climate change; therefore, they are highly subtle to perturbations in both terrestrial and aquatic environments because of their dual life histories, highly specialized physiological adaptations and specific microhabitat requirements (Blaustein 1994; Stebbins and Cohen 1995). Thus, they have been used as bioindicators of habitat quality. This high sensitivity of amphibians to habitat degradation and climate change has made amphibians the most endangered vertebrates on Earth (Catenazzi 2015). The amphibians' populations are rapidly declining worldwide due primarily to the loss and degradation of their natural habitats (Stuart et al. 2004; Mendelson et al. 2006; Wake and Vredenburg 2008; Ducatez and Shine 2017). Amphibians are threatened in part because of their low dispersal capacity and small home ranges (Blaustein 1994; Beebee 2005; Zeisset and Beebee 2008; Hillman et al. 2014).

We studied two *Ambystoma* species, *Ambystoma altamirani* and *Ambystoma rivulare*, which are micro-endemic mountain mole salamanders that inhabit slow-flowing streams within the TMVB (Lemos-Espinal 2003; Shaffer et al. 2008; Barriga-Vallejo et al. 2015). According to the IUCN Red List the conservation status of *A. altamirani* is Endangered (Shaffer et al. 2008a) and *A. rivulare* is Data Deficient (Shaffer et al. 2008b), according to Mexican law both species are endangered (SEMARNAT 2010), and their environmental vulnerability scores are 13 on a scale of 3 to 19. This score places them between medium and high vulnerability, primarily because of their restricted geographic and ecological distribution (Wilson et al. 2013; Lemos-Espinal et al. 2016; Woolrich-Piña et al. 2017). *Ambystoma altamirani* is mainly distributed in the Sierra de las Cruces and the Corredor Biológico Chichinautzin and *A. rivulare* is mainly distributed in the Nevado de Toluca Volcano and Reserva de la Biósfera Santuario Mariposa Monarca. These areas are subject to pressures such as legal and illegal logging (Sunny et al. 2019a, b; González-Fernández et al. 2019) the introduction of exotic species such as trout, human settlements and pollution of streams (Heredia-Bobadilla et al. 2016, 2017; Woolrich-Piña et al. 2017; Zamora et al. 2018). Although these areas present some category of protection such as in the case of the Nevado de Toluca Volcano, originally decreed as a National Park, this reserve was recently decreed as a Flora and Fauna Protected Area, a much less restrictive category that allows forest harvesting practices with commercial purposes, construction of eco-tourism sites and eco-tourism activities (Mastretta-Yanes et al. 2014; González-Fernández et al. 2019) which can increase the loss or disturbance of the habitat.

Therefore, we studied the genetic diversity and structure, effective population size, inbreeding and genetic bottlenecks of two populations of *A. rivulare* and A. *altamirani* in two sites of *Abies-Pinus* forest with little or no protection surrounded by two of the largest metropolitan areas in the country and the world. This information can be useful to help to raise conservation strategies for these micro-endemic mole salamander species.

Material and methods

Study area and population sampling

We sampled two populations of each species (Fig. 1B); the first population of *A. altamirani* was in Organillos (19°31'38.17"N, 99°28'39.92"W (the datum in all coordinates described are: WGS–84), with an altitude of 3,335 MASL), and the second population was in Sehuayán (19°31'31"N, 99°26'09.52"W with an altitude of 3,185 MASL), both of which are in Sierra

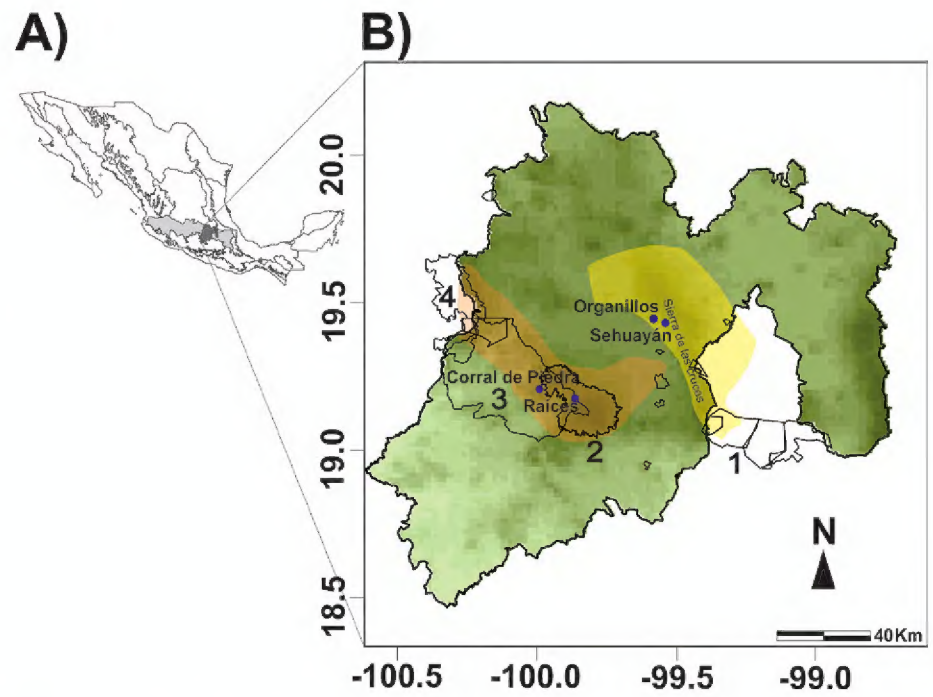

Figure 1. A) Map of Mexico showing in light grey the Trans-Mexican Volcanic Belt and in dark grey the State of Mexico. B) Map of the State of Mexico with an elevation raster, the darker areas refer to high elevation. The sampling sites are shown in blue and the polygons represented the natural protected areas: 1. The Corredor Biológico Chichinautzin, 2. Nevado de Toluca Volcano, 3. Zona Protectora Forestal Los Terrenos Constitutivos de las Cuencas de los Ríos Valle Bravo, Malacatepec, Tilostoc y Temascaltepec and 4. Reserva de la Biósfera Santuario Mariposa Monarca. The polygon in yellow represent the distribution of *Ambystoma altamirani* and in orange the distribution of *Ambystoma rivulare* according Woolrich-Piña et al. (2017).

de las Cruces. The first population of *A. rivulare* was in Corral de Piedra (19°13'6.60"N, 99°57'54.77"W, with an altitude of 2,836 MASL), and the second population was in Raíces (19°9'37.26"N, 99°49'32.11"W, with an altitude of 3,225 MASL), both of which are in the Nevado de Toluca Volcano (NTV). We sampled the individuals with a fishing net, and we sampled 2 mm^2 of tail clips of adult mole salamanders. This methodology is a low-impact method that does not affect the survival or growth of the mole salamanders (Arntzen et al. 1999; Polich et al. 2013). Tissue was preserved in 90% ethanol and then frozen at -20 °C until processed. Finally, we released the mole salamanders after tail clipping. Our study received the approval of the ethics committee from Universidad Autónoma del Estado de México (3047-2011E, 4732/2019CIB and 9855714) and the collection permits of SEMARNAT (SEMARNAT: SGPA/DGVS/001777/18).

Genetic analysis

We extracted DNA following the manufacturer's instructions for the GF-1 nucleic acid extraction kit (Vivantis Technologies, Subang Jaya, Malaysia), and we used it as a template for amplification of nine microsatellite loci following published protocols (Parra-Olea et al. 2007). PCR microsatellite products were multiplexed and run on an ABI Prism3730xl (Applied Biosystems, Foster City, CA, USA) with Rox-500 as an internal size standard. We obtained allele sizes with PEAKSCANNER 1.0 software (Applied Biosystems), and the fragment lengths were obtained with TANDEM 1.08 software (Matschiner and Salzburger 2009). In all runs we included negative controls in at least two runs and we repeat 2 samples per plate to account for genotyping error and to guarantee reproducibility.

Potential scoring errors and genotype accumulation curve

We tested the presence of null alleles and large allele dropout in the MICROCHECKER 2.2.3 software (Van Oosterhout et al. 2004). In addition, in POPPR 2.4.1 (Kamvar et al. 2014) for R software (version 3.4.0; R Development Core Team 2017), we made an analysis to create a genotype accumulation curve we used for determining the minimum number of loci necessary to discriminate between individuals in each population of the species studied (Kamvar et al. 2014). This function randomly samples loci without replacement and counts the number of observed multilocus genotypes (Kamvar et al. 2014).

Genetic diversity

All analyses were done for each study location (it was determined that the sampling sites were independent populations by the STRUCTURE results, see below) and species. We calculated the observed (H_o) and expected (H_e) heterozygosity, the number of alleles (N_a), effective number of alleles (N_e), number of genotypes and the number of heterozygotes and homozygote genotypes in STRATA G 2.0.2 (Archer et al. 2017) and GENALEX. We calculated departures from Hardy-Weinberg equilibrium (HWE) and Linkage Disequilibrium (LD) between pairs of microsatellite loci in PEGAS package (Paradis 2010) implemented in R. These calculations were evaluated for each sampled locality and locus with a Markov chain approximation considering 10,000 dememorizations, 1,000 batches and 10,000 iterations per batch. In order to correct the P values, we used a False Discovery Rate (FDR) approach according to Benjamini and Hochberg (1995) implemented in the package FDRTOOL 1.2.15 (Strimmer 2008; Klaus and Strimmer 2013) for R.

Genetic structure

We searched for a genetic structure pattern using several algorithms for each of the species and sampling sites. First, we used a Bayesian algorithm implemented in the STRUCTURE 2.3.4 software (Pritchard et al. 2000; Falush et al. 2003; Hubisz et al. 2009). The chosen parameters were correlated allele frequencies with 1,000,000 burn-in periods and 1,000,000 MCMC iterations (Falush et al. 2003). For the degree of admixture, a Dirichlet parameter was applied with correlated allele frequencies. Therefore, the most credible number of populations were estimated using the maximum value of ΔK (Evanno et al. 2005), applied in the STRUCTURE HARVESTER 0.6.92 software (Earl and vonHoldt 2012). The second method was the analysis of molecular variance (AMOVA) based on F_{ST} and R_{ST} as implemented by GENALEX 6 (Peakall and Smouse 2006). A Wilcoxon test with 30,000 permutations was applied to search significance, using the degree of similarity of the populations based on the populations' genotypes in GENALEX 6. In order to detect the similarity degree of the populations of each species, we applied a Discriminant Principal Components Analysis (DPCA) based on the genotypes; we used the populations as priors and we ran the analysis in ADEGENET 2.0.1 (Jombart et al. 2016) and ADE4 1.7-6 (Dray and Siberchicot 2017) packages for the R software, and we calculated F_{ST} based on Weir and Cockerham (1984) in GENALEX 6. Finally, we tested the existence of the population structure by computing Minimum Spanning Networks (MSN) with Bruvo's (Bruvo et al. 2004) and Nei´s distance algorithm (Nei 1972) with 1,000 bootstraps in POPPR 2.4.1 and MAGRITTR 1.5 (Bache and Wickham 2016) for the R package. This analysis visualizes the relationships among individuals and it can be a more adequate visualization tool than trees (Bache and Wickham 2016).

Genetic bottlenecks, effective population size and relatedness

The historical signal of demographic fluctuations was explored for each population by applying a Bayesian algorithm implemented in MSVAR 0.4.1 software (Beaumont 1999). We estimated the rate of change (r) of the effective population size, defined as N_{crnt}/N_{stbl} (where N_{crnt} was the current inbreeding effective population size and N_{stbl} was the ancestral stable inbreeding effective population size). The r ratio was expressed in log10. Therefore, the population declined if we had a negative r value, was stable if r is equal to zero, and the population was expanded if the r value was positive (Gasca-Pineda et al. 2013; Sunny et al. 2015). In order to test for a genetic signature of recent bottlenecks, we used the BOTTLENECK 5.1.26 software (Cournet and Luikart 1996; Piry et al. 1999). We estimated the observed and expected heterozygosity under the two-phase model (TPM) because the TPM is an intermediate model of evolution which is considered more appropriate for microsatellites. The settings applied were for a 90% step-wise mutation model and 10% variance and were run with 10,000 replicates. Excess heterozygosity was tested using a Wilcoxon test. In order to explore the actual effective population size (N_e), we used the LD method implemented in the NEESTIMATOR 2 software (Do et al. 2014). We calculated the F_{IS} inbreeding values in GENALEX 6. Also as an inbreeding measure, we used the relatedness estimator (rqg) of Queller and Goodnight (1989), which was calculated by the GENALEX software. To test for significant differences among mean population relatedness, we calculated the upper and lower 95% confidence intervals for the expected range of rqg using 9999 permutations. These intervals corresponded to the range of rqg that would be expected if reproduction was random across populations. Additionally, we calculated confidence intervals for estimates of mean relatedness within a population to 95% by bootstrap resampling (9999 permutations). Population rqg values that fall above the 95% expected values indicate that processes such as inbreeding or genetic drift are increasing relatedness. Finally, relatedness among individuals was evaluated using the ML-RELATE software (Kalinowski et al. 2006).

Results

Population sampling

One hundred and fifty-two individuals were sampled from two *Ambystoma* species (*A. altamirani* and *A. rivulare*), two locations were sampled and 38 tissues were collected from each locality.

Potential scoring errors

We did not find evidence of null alleles or large allele dropout in the populations of each species. The genotype accumulation curve found that the minimum number of loci necessary to discriminate between individuals was eight (Suppl. material 1: Fig. S1). Therefore, we concluded that our study had enough loci (N = 9).

Genetic diversity

Across the nine loci in the *A. altamirani* populations in Organillos we found 3–7 alleles per locus and a total of 30 alleles; Sehuayán had 2–7 alleles per locus with a total of 26 alleles (Table 1, Suppl. material 1: Fig. S2). In Corral de Piedra the first *A. rivulare* population we found 2–5 alleles per locus with a total of 20 alleles, and in Raíces the second *A. rivulare* population we found 2–6 alleles per locus with a total of 33 alleles (Table 1, Suppl. material 1: Fig. S3). In the *A. altamirani* Organillos population we found 29 homozygote genotypes and 35 heterozygote genotypes, In the second *A. altamirani* population Sehuayán we found 16 homozygote genotypes and 22 heterozygote genotypes. In the *A. rivulare* Corral de Piedra population we found 26 homozygote genotypes and 19 heterozygote genotypes, and Raíces had 21 Homozygote genotypes and 20 heterozygote genotypes (Suppl. material 1: Table S1). In relation to the observed and expected heterozygosity values, the *A. altamirani* Organillos population showed lower observed heterozygosity values (H_o = 0.719±0.033) compared to Sehuayán (H_o = 0.857±0.029); the *A. rivulare* population Corral de Piedra showed lower observed heterozygosity values (H_o = 0.576±0.034) as compared to Raíces (H_o = 0.754±0.059; Table 1). False

Table 1. Genetic diversity values in the four *Ambystoma* populations studied based on nine microsatellite loci. N: sample size, Na: number of alleles, Ne: number of effective alleles, A: allelic richness, H_o: observed heterozygosity, H_e: expected heterozygosity.

Species	Population	N	Na	Ne	A	Ho	He
Ambystoma altamirani	Organillos	38	5.222	3.623	0.137	0.719	0.706
	Sehuayán	38	4.222	3.071	0.111	0.857	0.636
	Total mean	38	4.772	3.347	0.124	0.788	0.671
	SE	0	0.449	0.237	0	0.027	0.026
Ambystoma rivulare	Corral de Piedra	38	3.333	2.513	0.088	0.576	0.562
	Raíces	38	3.889	2.815	0.102	0.754	0.617
	Total mean	38	3.611	2.664	0.095	0.665	0.589
	SE	0	0.293	0.193	0	0.039	0.029

discovery rate correction tests found departures from HWE due to heterozygote deficiency in one locus in the populations of Organillos (*A. altamirani)*, Corral de Piedra (*A. rivulare)* and Raíces (*A. rivulare)* (Suppl. material 1: Table S2). We did not find LD between any loci of either population for each species.

Genetic structure

Bayesian assignment analyses corroborated high population divergences among populations (Fig. 2). The highest log likelihood given by STRUCTURE and ΔK method was K = 2 (LnPr = -1793.2) for *A. altamirani* and K = 2 (LnPr = -1457.6) for *A. rivulare*. The populations of *A. altamirani* present admixia with medium genetic differentiation among them (F_{ST} = 0.053; Table 2), but the populations of *A. rivulare* do not present admixia with high genetic differentiation among them (F_{ST} = 0.211; Table 2). In relation to the AMOVA results, for *A. altamirani* the results revealed the majority of genetic variation resided within populations (92%) followed by among populations (8%), with an F_{ST} fixation index of 0.084 and a p-value = 0.001, for *A. rivulare* the AMOVA results showed significant levels of genetic variation within populations (67%) and among populations (33%), with an F_{ST} fixation index of 0.332 and a p-value = 0.001(Suppl. material 1: Tables S3, S4). The DPCA and the MSN found the same patterns of population structure where the populations of *A. altamirani* are more similar among them, whereas the populations of *A. rivulare* are very different among them (Figs 3, 4).

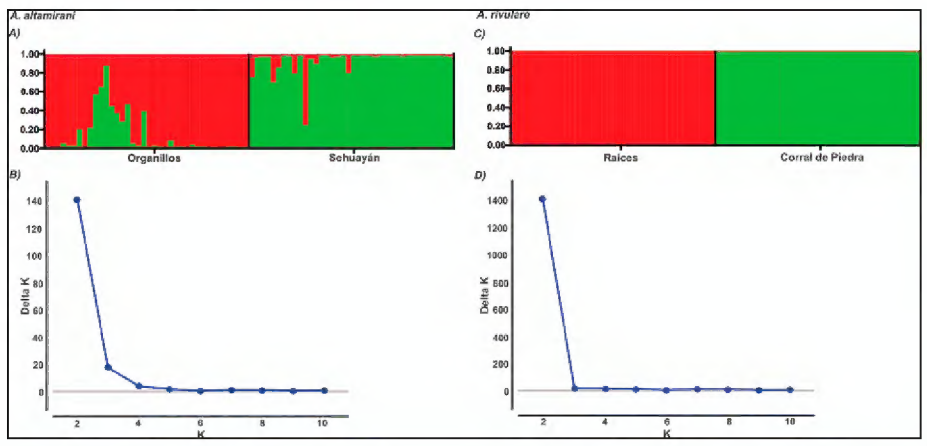

Figure 2. A) Population genetic structure of *Ambystoma altamirani* analyzed with STRUCTURE. B) Evanno et al. (2005) plots for detecting the number of K groups that best fit the data. C) Population genetic structure of *Ambystoma rivulare* analyzed with STRUCTURE. D) Evanno et al. (2005) plots for detecting the number of K groups that best fit the data.

Table 2. F_{IS}, F_{ST} and F_{IT} fixation indices estimated according to Weir and Cockerham (1984) in the four *Ambystoma* populations studied.

Species		F_{IS}	F_{IT}	F_{ST}
Ambystoma altamirani	**Total mean**	-0.190	-0.128	0.053
	SE	0.049	0.052	0.012
Ambystoma rivulare	**Total mean**	-0.141	0.095	0.211
	SE	0.051	0.079	0.045

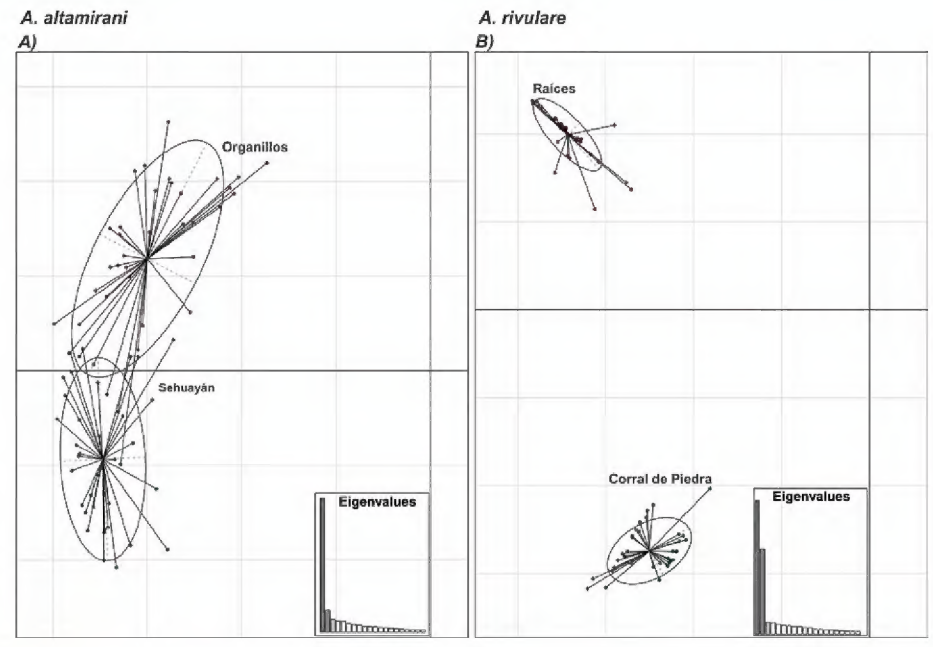

Figure 3. Scatterplot showing the relationships among the populations of each species based on a Discriminant Principal Components Analysis of 9 microsatellite genotypes.

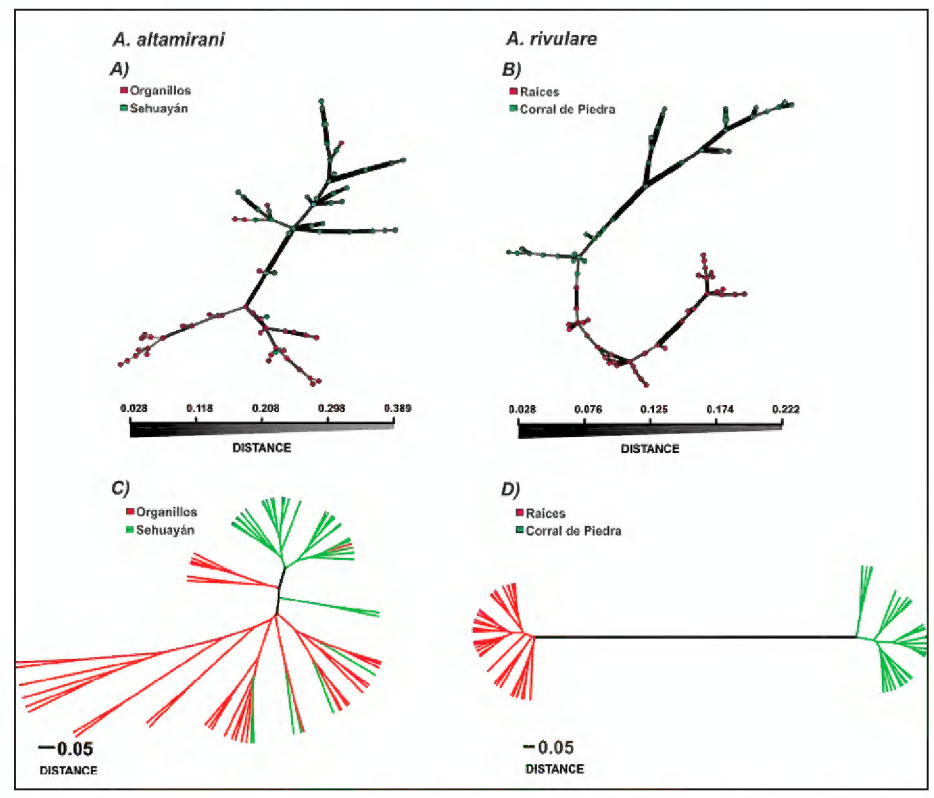

Figure 4. A) and B) Minimum Spanning Networks with the Bruvo's distance algorithm, representing the relationships among individuals and populations of each species. C) and D) Tree constructed by the NJ method using the estimated standardized genetic distances using the Nei´s distance algorithm (Nei 1972) with 1,000 bootstraps.

Genetic bottlenecks, effective population size and relatedness

MSVAR results suggested that there has been a significant population size reduction in all the studied *Ambystoma* populations: Organillos, r = -0.972; Sehuayán, r = -1.403; Corral de Piedra, r = -1.031 and Raíces, r = -1.399. The bottleneck analysis detected genetic signs of recent demographic changes typical of bottleneck events, associated with a heterozygote excess in all populations: Organillos, P = 0.008; Sehuayán, P = 0.002; Corral de Piedra, P = 0.002; Raíces, P = 0.002 and Corral de Piedra and Sehuayán had a shifted distribution. The effective population size (N_e) estimated from LD was N_e = 34.7 (20.9–21.7, 95% CI) for Organillos, N_e = 44.1 (21.3–36.0, 95% CI) for Sehuayán, N_e = 57.6 (21.3–37.6, 95% CI)

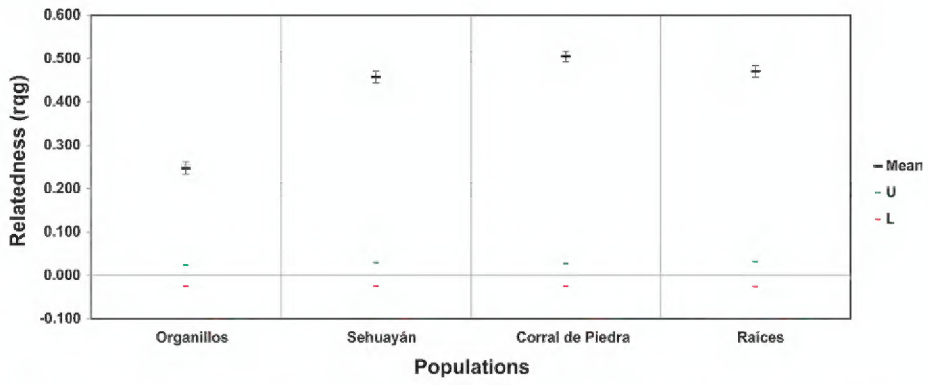

Figure 5. Mean within-lake pairwise relatedness coefficient rqg across the four *Ambystoma* populations studied. The green bars are 95% upper and lower expected values for a null distribution generated from 9999 permutations of data from all populations, and enclose the values expected if breeding were panmictic across all populations; relatedness in all sampled populations fell outside the range expected under panmixia. Black bar represents the observed mean relatedness in each population, the black bars are the upper and lower bootstrap value for each population.

for Corral de Piedra and N_e = 41.5 (20.1–23.6, 95% CI) for Raíces. The F_{IS} statistic as an indicator of inbreeding for the *A. altamirani* populations showed negative and low inbreeding values (F_{IS} = -0.128; Table 2) and showed positive, low inbreeding values for the *A. rivulare* populations (F_{IS} = -0.095; Table 2). We found that mean pairwise relatedness (r) within populations (Fig. 5) was generally in accordance with that observed in other *Ambystoma* populations (Parra-Olea et al. 2012; Sunny et al. 2014a; Percino-Daniel et al. 2016). Organillos had low values of inbreeding [mean rqg = 0.247, confidence interval (CI) = 0.024 -(-0.025)], Sehuayán had medium to high levels of inbreeding [mean rqg = 0.458, confidence interval (CI) = 0.029 -(-0.025)], Corral de Piedra had the highest inbreeding values [mean rqg = 0.505, confidence interval (CI) = 0.028 -(-0.026)] and Raíces had medium to high inbreeding values [mean rqg=0.471, confidence interval (CI) = 0.032 -(-0.026). In all the populations, the rqg values fell above the 95% expected values from permutations which indicates that inbreeding or drift are increasing the relatedness; they also fell outside the range expected under panmixia. Furthermore, there were low values of inbreeding in the relatedness analysis, the proportion of relatedness of individuals within each population was similar and most of the individuals were unrelated, followed by full siblings, half-siblings and parents/offspring (Suppl. material 1: Table S5).

Discussion

In the present study, we found medium to high levels of genetic diversity expressed as heterozygosity in both species and all the populations (H_o = 0.576–0.754). Also, the two-species presented few alleles per locus (2–7 alleles per locus) and genotype (Suppl. material 1: Table S1). However, the microsatellites used were developed for other *Ambystoma* species; this non-specificity could be the reason why we found the low number of alleles. Each sampled locality represents a population with a significant level of genetic structure. The effective population size was small in both species, but it is similar to other mole salamanders with restricted distributions or with recently fragmented habitats (Parra-Olea et al. 2012; Sunny et al. 2014a; Percino-Daniel et al. 2016; Heredia-Bobadilla et al. 2017). These results are important in order to design better management and conservation strategies to avoid the extinction of these micro-endemic species of the TMVB.

Genetic diversity

The observed heterozygosity values were medium to high, and most of the genotypes were heterozygous with the exception of the two populations of *A. rivulare (*Corral de Piedra and Raíces) (Table 1, Suppl. material 1: Table S1). *Ambystoma* species have high levels of genetic diversity (Goprenko et al. 2007; Greenwald et al. 2009; Sunny et al. 2014a; Percino-Daniel et al. 2016) despite having a fragmented and limited distribution. However, the human population growth in the towns around the two populations of *A. rivulare* (Corral de Piedra and Raíces) has been increasing in recent decades, causing an influx of sewage, waste from local agriculture and pollutants from trout farms into the rivers. Also, in Corral de Piedra we did not find larvae, and each mole salamander had tail bites. This could be due to trout predation (Zamora et al. 2018) because of trout escape from farms, which favors native species reduction (Gamradt et al. 1997; Kiesecker and Blaustein 1997; Kiesecker et al. 2001). Introduced fish species have been linked to reductions in amphibians' population sizes (Pearson and Goater 2009; Zambrano et al. 2010; Alcaraz et al. 2015; Zamora et al. 2018), sometimes to the point of extinction from direct consumption (Watson et al. 1991; Tyler et al. 1998) increasing the loss of genetic diversity. We found a significant deviation from the HWE proportions due to a heterozygote deficiency in Organillos, Corral de Piedra and Raíces. This is a common result when microsatellites are not specific for the species and for threatened species with fragmented populations (Degne et al. 2007; Spear and Storfer 2010; Vázquez-Domínguez et al. 2012; Sunny et al. 2014a).

Genetic structure

Structure analysis suggests two populations for each species. The populations of *A. rivulare* showed no signs of admixia, although the populations of *A. altamirani* were more admixed (Figs 2–4). The populations of *A. altamirani* and *A. rivulare* we studied are ~67 km apart in linear distance, and the two populations of *A. rivulare* are ~16 km apart in linear distance, so the genetic exchange is extremely reduced. The two populations of *A. altamirani* are closer, ~2.5 km apart in linear distance so gene flow could still take place. In other studies, mole salamander

migrations occur between temporal ponds and lakes as individuals look for food-rich habitats (Percino-Daniel et al. 2016). The known maximum dispersal distance in mole salamanders is less than 2 km (Smith and Green 2005; Percino-Daniel et al. 2016). Migration among other populations could be limited as a result of physical barriers like discontinuity of rivers, forests, roads, and towns, as well as the strong philopatric tendencies of the mole salamanders for breeding sites favoring high genetic structuring, even at small scales (Spear et al. 2005; Zamudio and Wieczorek 2007; Richardson 2012; Richardson and Urban 2013). These populations in the TMVB occupy some of the most disturbed areas of the country, mainly due to habitat fragmentation stream pollution and fish introduction (Recuero et al. 2010; Zambrano et al. 2010; Rodríguez-Amador et al. 2013; Lemos-Espinal et al. 2016). Since there are different factors affecting *Ambystoma* populations, we recommend some measures to try to minimize the impact of the anthropogenic activities on these amphibian populations.

Genetic bottlenecks, effective population size and relatedness

The studied populations are isolated from other populations of mole salamanders. This phenomenon could explain the low N_e values (N_e = 34.7–57.6) found in all populations and the asymmetry in the proportions of males and females and differences in the reproductive success between individuals favour low N_e values (Wang 2009; Savage et al. 2010; Parra-Olea et al. 2012; Percino-Daniel et al. 2016) due to high asymmetry in reproductive success among members of a population (Savage et al. 2010). Some years, only a few individuals successfully breed, and the variance in mating success may contribute strongly to lower overall effective population sizes (Savage et al. 2010). Another explanation of the low N_e values found could be the bottleneck effect caused by the introduction of trout; however, we do not know when this introduction happened, as trout predate the early stages of development of *Ambystomas* and also eat the tail of adult *Ambystomas*, a phenomenon which has already been reported by other studies (Pilliod and Peterson 2001; Welsh et al. 2006; Zambrano et al. 2010; Martín-Torrijos et al. 2016). It has also been reported that trout can transmit pathogens and certain emerging infectious diseases (Johnson and Speare 2005; Fernández-Benéitez et al. 2008; Van den Berg et al. 2013; Sandoval-Sierra et al. 2014). Likewise, trout can compete for food with *Ambystomas* (Werner and Anholt 1996; Tyler et al. 1998). All these features can lead the population to a process of genetic bottleneck or genetic drift, which in turn reduces genetic diversity and makes the population lose fitness and the ability to adapt to changes in the environment (Frankham et al. 2005). In all populations, the rqg values were above the 95% expected values from permutations (Fig. 5), indicating that inbreeding or genetic drift are increasing the relatedness, and they fell outside the expected range under panmixia. Furthermore, there were low values of inbreeding in the relatedness analysis; the proportion of relatedness of individuals within each population was similar. Despite the lack of strong signs of inbreeding and relatedness, inbreeding and genetic drift are acting in these populations.

Conservation implications

In order to conserve this species and all the species that live in the coniferous forests of TMVB, it is necessary to avoid excessive legal and illegal logging and give support to the local communities with incentives such as payments for ecosystem services. Also, we consider the implementation of an environmental education program to be fundamental to avoid excess logging; maintaining and increasing forest core areas in order to minimize the forest edges, also, preventing the loss of the largest forest patches in order to avoid deviations from circularity in patch shapes to increase the area of core habitat (Ewers and Didham 2006; González-Fernández et al. 2019). Therefore, we must conserve the endangered *Abies-Pinus* forest. In the TMVB, there are only 1346.9 km^2 of *Abies* forest (1.1%) and 6507.7 km^2 of *Pinus* forest (5.4%). It is therefore extremely important to conserve these forests, considering that Mexico had the seventh-highest net annual forest loss of any country in the world, and the clearing of primary forests averaged more than 1 percent per year (FAO 2011; Blackman et al. 2015), placing Mexico in the first levels of global deforestation (FAO 2006; Ellis and Porter-Bolland 2008). Likewise, the amphibians and reptiles have a very limited distribution, sometimes in very small areas with special characteristics that generate certain microhabitats suitable for their survival and reproduction (Sunny et al. 2014b; Lemos-Espinal et al. 2016, 2017; Zamora et al. 2018). So, it is necessary to start considering the creation of natural reserves with landscape corridors that include these microhabitats. Also, it is important to implement better reforestation and assisted regeneration practices, with germplasm of the area. Currently, it is still a common practice that authorities reforest the areas of *Abies* forests with other conifer species, such as *Pinus ayacahuite*, *P. pseudostrobus*, *P. patula* or *Cupressus lusitanica*, which in turn can change the microclimate and the environmental and habitat conditions that the *Abies* forest generates, as high humidity and low temperatures, characteristics that the high-mountain *Ambystomas* are adapted to (Sunny et al. 2014b; Monroy-Vilchis et al. 2015; Lemos-Espinal et al. 2016). This is important to highlight since, with the recent change of protection level of the NTV forest, harvesting practices are being allowed in almost all *Abies* forest extensions (Mastretta-Yanes et al. 2014; González-Fernández et al. 2019). Likewise, it is necessary to stop the influx of sewage and decrease waste from local agriculture and pollutants into the rivers, avoid the excess of free livestock, grassland fires, restrict ecotourism activities and prohibit the use of

Ambystoma salamanders as traditional medicine for the lungs or traditional food (Monroy-Vilchis et al. 2015). It is also necessary to have more control over trout breeding because trout escape from farms to the streams, primarily due to low-quality fences and meshes dividing the farms from streams. We recommend divisions to be built higher and periodically revised to avoid holes from which trout can escape. Trout farms are affecting the populations of mole salamanders; several studies have demonstrated that the predation by introduced fish has been linked to reductions in the population's size, survival, growth, egg predation, reproduction and organic waste pollution of the rivers (Valiente 2006; Mercado-Silva et al. 2008; Drake et al. 2014; Kenison et al. 2016) since mole salamanders do not recognize trout as predators and their survival decreases proportionately (Petranka et al. 1987; Petren et al. 1993; Relyea 2001; Pilliod et al. 2013). Trout farms have been encouraged by Mexican governmental agencies and recommended as a potential conservation tool for native forests (López-García et al. 2014; Zamora et al. 2018), so the best strategies may include isolating trout farms from streams containing native amphibians, increasing efforts to prevent trout escaping and attempting to eradicate populations of escaped trout from streams, thereby balancing the economic and conservation value of trout farms with their potential negative effects on native amphibians (Zamora et al. 2018). Also, it is necessary that each trout farm has an area of wastewater treatment since these waters when released to the stream can change the physical, chemical and bacteriological parameters and contaminate the stream for up to 3–12 km (Boaventura et al. 1997; Johnson and Speare 2005). Certain sediments turn completely anoxic and, with hydrogen sulfide by-products of microbial sulfate reduction, can increase to toxic levels (Valdemarsen et al. 2009, 2010, 2015). Also, it is very important that management agencies restore a fishless state in all lentic habitats that have the potential to host mole salamanders (Pearson 2004). Finally, additional research is needed, as well as effective communication between scientists and managers and sensible management actions to carry out a coordinated strategy between scientists, managers, local people and fishermen to ensure the long-term conservation of the mole salamanders. If these conservation strategies are not carried out, the expected heterozygosity values found will continue to decline, inbreeding will be higher, the effective population size will be lower and the mole salamanders will not have enough genetic diversity to be able to adapt to changes in the environment (Lande 1988; DeYoung and Honeycutt 2005; Frankham et al. 2005; Hedrick 2011).

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Supplementary material 1

Complementary figures and tables that support the results found in this study.

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Data type: Multimedia.

Explanation note: **Figure S1.** Genotype accumulation curve to determine the minimum number of loci necessary to discriminate between individuals in a population. **Figure S2.** Allelic frequencies of the nine loci in the two Ambystoma altamirani populations studied. **Figure S3.** Allelic frequencies of the nine loci in the two Ambystoma rivulare populations studied. **Table S1.** Number of genotypes in the four Ambystoma populations studied. **Table S2.** Hardy-Weinberg and inbreeding coefficients of Weir and Cockerham (W & C) for the four Ambystoma populations studied, values in bold were significant deficiency of heterozygosity ($p \leq 0.05$) with the FDR correction. **Table S3.** Analysis of molecular variance based on FST values for the populations of Ambystoma altamirani. **Table S4.** Analysis of molecular variance based on FST values for the populations of Ambystoma rivulare. **Table S5.** Genetic relationships in the populations in the four Ambystoma populations studied.

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